et al. (U.S. Patent No. 5,112,611), Nakayama et al. (U.S. Patent No. 5,531,917) and Weiner et al. (U.S. Patent No. 5,171,737).

In a telephone interview between the undersigned and Supervisory Primary Examiner Padmanabhan on January 22, 2003, it was agreed that the §103 rejection outstanding in this application would be overcome by amending the claims to include a recitation to the effect that the block copolymer constituent of the claimed composition is not cross-linked. It was recognized by Supervisory Primary Examiner Padmanabhan that such an amendment is consistent with the arguments for patentability advanced by applicants during the course of prosecution of these applications.

A Notice of Allowability was issued February 7, 2003, including an Examiner's Amendment that essentially confirmed the outcome of the above-mentioned telephone interview.

In accordance with the amendment submitted herewith, claim 1 of the present application has been amended by the additional recitation that the "block copolymer constituent is not cross-linked to form networks". This amendment is in keeping with the agreement reached between the undersigned and Supervisory Primary Examiner Padmanabhan, as noted above, and finds support in the present specification, at page 6, line 3 through page 7, line 15. As a result of this amendment, it is believed that the claimed invention is clearly distinguishable over the combined disclosures of Hubbel et al., Ahmad, Nakayama et al. and Weiner. In this regard, it is noted that the

photopolymerizable, biodegradable hydrogels of Hubbel et al. are adapted to be cross-linked so as to form networks. See Column 5, lines 15-64 and Column 8, lines 5-27 of Hubbel et al.

Applicants wish to take this opportunity to take exception once again the Examiner's assertion that the disclosures of Hubbel et al., Ahmad et al., Nakayama et al. and Weiner et al., in and of themselves, provide the motivation required for one of ordinary skill in the art to arrive at applicants' invention.

Although Nakayama et al. may reasonably be viewed as providing a solution to the purported problem of Bioprase instability in contact lens cleaning compositions, it does not reasonably provide any suggestion or motivation for those skilled in the art to similarly attempt to stabilize papain, in the event that papain were incorporated in the biodegradable hydrogels of Hubbel et al., as proposed by the Examiner. The data provided in Nakayama et al. pertain exclusively to the stabilizing effect of certain surfactants on Bioprase in a contact lens cleaning composition. Bioprase is a proteolytic enzyme derived from a microorganism of the genus Bacillus. See Column 3, lines 20-25 of Nakayama et al. There is no data set forth in Nakayama et al. that tends to show that papain would exhibit similar instability in a contact lens cleaning composition. Indeed, as noted in the attached page from the SERVA Electrophoresis GmbH web site, papain is "known for its unusually high stability" and "as crystalline suspension in NaCl solution at near neutral pH,

papain can be stored for many months at 4°C without any significant loss of activity". The conditions under which papain exhibits such stability are comparable to the conditions specified in Table 1 of Nakayama et al.

In any event, maintaining proteolytic enzyme stability is certainly not regarded as a problem in the case of the biodegradable hydrogels of Hubbel et al., with which papain is proposed to be combined. It is noteworthy in this connection that Hubbel et al., at Column 16, lines 50-52 disclose that the water present in the hydrogels described therein "can be expected to help proteins and enzymes entrapped in such gels in maintaining their native conformation and reducing deactivation". Thus, rather than promoting enzyme instability, the water content of their hydrogels is considered by Hubbel et al. as beneficial for maintaining enzyme conformation and reducing deactivation. This is not merely theoretical conjecture by Hubbel et al. On the contrary, the compatibility between proteolytic enzymes and the hydrogels of Hubbel et al. was demonstrated in practice. As stated at Column 21, lines 33 and 34 of Hubbel et al., "[f]ully active tPA can be released for periods up to at least two (2) months.

In summary, there is plainly no motivation provided in the disclosures of Hubbel et al., Ahmad et al., Nakayama et al. and Weiner et al. for combining them in the manner proposed by the Examiner, and even if combined, the claims presented herewith are clearly patentably distinguishable over the resulting

combination.

Accompanying this submission is an Information Disclosure Statement listing references which the Examiner is respectfully requested to consider and make of record in this application.

In view of the present submission, all of the claims now pending in this application are believed to be in condition for allowance. Accordingly, the issuance of a Notice of Allowance is in order, and such action is earnestly solicited.

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Enclosure: Papain (from SERVA Electrophoresis GmbH web site - 2 pages)

Marked-Up Version of Replacement Paragraph

Pursuant to 35 U.S.C. §202(c), it is hereby acknowledged that the U.S. Government has certain rights in the invention described herein, which was made in part with funds from the National Science Foundation under Grant No. DMR-9502807.

Marked-Up Version of the Amended Claim

1. (Amended) A composition of matter comprising a therapeutic or diagnostic agent and a supremolecular complex, said complex comprising as constituents (i) a block copolymer, having at least one nonionic, water soluble segment and at least one polyionic segment, and (ii) at least one charged surfactant having hydrophobic groups, the charge of said surfactant being opposite to the charge of the polyionic segment of said block copolymer, wherein the block copolymer constituent is not crosslinked to form networks, the constituents of said complex [being] are bound by interaction between said opposite charges and between surfactant hydrophobic groups, and with the proviso that when said therapeutic or diagnostic agent is an ionic substance having a net charge opposite to the charge of said block copolymer, the net charge of said therapeutic or diagnostic agent is no more than 10.

PAPAIN

EC 3.4.22.2

Cat. nos. 31600, 31610

Structure:

Papain is an endolytic cysteine protease which is isolated from papaya latex. It consists of a single, folded polypeptide chain of 212 amino acid residues containing 3 disulphide bonds and one free functional -SH group at the active site . The three-dimensional structure has been elucidated (1, 2).

Specificity:

Papain is noted for its wide specificity. It preferentially cleaves peptide bonds involving basic amino acids, particularly arginine, lysine and residues following phenylalanine. It also has an esterase activity (1, 4).

Physical and Chemical Properties:

M_r:	ca.23,400 (3)
Optimum pH:	6 - 7
Optimum temperature:	65 °C
Isoelectric point:	8.75 (4) and 9.6 (5)

Activators:

In its native state, papain exhibits very low activity, as its free sulfhydryl group appears to be blocked. Mild reduction is therefore required for full activity. This can be achieved by means of reducing agents such as cysteine, glutathione (GSH) or HCN. Optimum activation can be achieved by the addition of 5 mM cysteine and 2 mM EDTA (6).

Inhibitors:

Hg++, Pb++ and other heavy metal ions (6). SH reagents like N-Ethylmaleimide, Iodoacetate (6), H_2O_2 (7), E-64, Leupeptin, PMSF, TPCK, TLCK (6), α 2-macroglobulin.

Unit definition:

1 U catalyses the hydrolysis of 1 µmol BAEE per minute at 25 °C, pH 6.2.

Stability and storage:

Papain is known for its unusually high stability and resistance against heat, organic solvents and reagents which cause denaturation of other enzymes. This resistance is however pH dependent. At a pH < 2, papain is rapidly and irreversibly inactivated. Kinetic studies can be performed over the pH range of 2.8 – 10.8 (8). As Lyophilized powder or as crystalline suspension in NaCl solution at near neutral pH, papain can be stored for many months at 4° C without any significant loss of activity (1, 6). In the activated form, the enzyme looses however 1-2% of its activity per day, probably due to autolysis or oxidation. Papain is resistant against 8 M urea (9), and tolerates exposure to many organic solvents. It exhibits unusual thermal stability , the dry enzyme being able to withstand temperatures of 100 °C for 3 hours (1, 6).
Papain is only incompletely soluble in water and glycerol and practically insoluble in most organic solvents.

Applications:

Papain can be used for the digestion of proteins e g. in the isolation of receptors (10), and in the structural investigation of proteins by means of limited digestion (11). It has also been used in proteinase-catalyzed peptide bond formation (12, 13). Technical applications: for recovery of silver from photographic films; in food industry for tenderizing meats and clearing beverages; in leather industry for bating skins and in bacteriology to produce peptons. Papain is also used for the removal of protein deposits from the surface of soft contact lenses.

Assay method:

The activity of papain is estimated by its ability to cleave N-benzoyl-L-arginine ethyl ester (BAEE). Free carboxylic groups are then measured by titration with NaOH (6).

Papain from Carica papaya ca.30 U/mg protein,2 x cryst. suspension in 0.05 M sodium acetate, pH 4.5, cont. 0.2 M NaCl	**Cat. No.** 31600.01	**Size** 50 mg
Papain from Carica papaya 02-0.4 U/ mg, lyophilized	**Cat. No.** 31610.04	**Size** 100 g

References:

1. Glazier, A.N. & Smith, E.L. (1971) In: The Enzymes (Boyer, P.D., ed.) 3rd edition, Vol. III, Academic Press,501-46. Papain and other plant sulfhydryl proteolytic enzymes.
2. Drenth, J., Jansonius, J.N., Koekoek, R. and Wolthers, B.G. (1971) In: The Enzymes (Boyer, P.D., ed.) 3rd edition, Vol. III, Academic Press, 485-99. Papain, X-ray structure.
3. Mitchel, R., Chaiken, I.M., and Smith, E.L. (1970) J. Biol. Chem. 245, 3485. The complete amino acid sequence of papain. Additions and corrections.
4. Smith, E.L. and Kimmel, J.R. (1960) in The Enzymes (Boyer, P.D. et al. eds.) 2nd edition, Vol. 4, Academic Press, 133- 73. Papain.
5. Sluyterman, L.AE. & DeGraaf, M.J.M. (1972) Biochim. Biophys. Acta 258, 554. The effect of salts upon the pH dependence of the activity of papain and succinyl-papain.
6. Arnon, R. (1970) In: Methods Enzymol. (Perlman, G.E. & Lorand L., eds.) Vol. 19, 226-44. Papain.
7. Lin, W.S., Armstrong, D.A., and Gaucher, G.M. (1975) Can. J. Biochem. 53, 298. Formation and repair of papain sulfenic acid.
8. Stockell, A. & Smith, E.L. (1957) J. Biol. Chem. 227, 1.
9. Sluyterman, L.AE. (1967) Biochim. Biophys. Acta 139, 418
10. Kouvonen, I. (1980) Biochem. Biophys. Acta 626, 244-53. Solubilization of the pig ileal intrinsic factor receptor with papain treatment and studies on the solubilized receptor.
11. Matsumura, S. Kumon, A. and Chiba, T. (1985) J. Biol. Chem.260 (3),1959-66. Proteolytic substructure of brain myosin.
12. Kullmann, W. (1984) Biochem . J.220,405- 16. Kinetics of chymotrypsin-and papain-catalysed synthesis of (leucine)enkephalin and (methionine)enkephalin.
13. Kullmann, W. (1982) Proc. Natl. Acad. Sci. USA 79, 2840-4. Protease-catalyzed peptide bond formation: application to synthesis of the COOH-terminal octapeptide of cholecystokinin.

Related Information and Products

Substrates for Papain